

We claim:

- 1 A method for derivatization of hydroxy amino acids in a peptide or a protein which contains one or more hydroxyamino acid residues which comprises the steps of:

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providing a peptide or a protein comprising one or more hydroxy amino acid residues wherein the hydroxy group of at least one of the hydroxy amino acid residues in the peptide or protein is protected with an azide-bearing protecting group and other potentially reactive groups in the peptide or protein are protected with one or more protecting groups that are not azide-bearing protecting groups;

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deprotection of the hydroxy amino acid residue of one or more azide protected hydroxy groups in the peptide or protein under conditions that remove the azide-bearing protecting group, but do not substantially remove the one or more other protecting groups of the peptide or protein; and

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functionalization of the free hydroxy group to generate a modified peptide or protein.

2. The method of claim 1 wherein the functionalization step is selected from the group consisting of sulfation, phosphorylation and glycosylation.

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3. The method of claim 1 wherein the hydroxy amino acid residue is a serine.

4. The method of claim 1 wherein the hydroxy amino acid residue is a threonine.

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5. The method of claim 1 wherein the hydroxy amino acid residue is a tyrosine.

6. The method of claim 1 wherein the azide-bearing protecting group is an azidomethylene group.

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7. The method of claim 1 wherein the azide-bearing protecting group is removed under reducing conditions.
8. The method of claim 7 wherein the azide-bearing protecting group is removed by treatment with stannous chloride.
9. The method of claim 1 wherein the protected peptide or protein is provided by peptide synthesis employing an amino protecting group and one or more side chain protecting groups.
10. The method of claim 9 wherein the protected peptide or protein is synthesized by a method comprising solid phase peptide synthesis.
11. The method of claim 10 wherein solid phase peptide synthesis is performed using the Fmoc protecting group as the amino protecting group.
12. The method of claim 10 wherein solid-phase peptide synthesis is performed using the Boc protecting group as the amino protecting group.
- 20 13. The method of claim 1 wherein the protected peptide or protein further comprises one or more hydroxy amino acid residues that are protected with a protecting group that is not an azide-bearing protecting group and is removed under conditions that are substantially orthogonal to those used to remove the azide-bearing protecting group.
- 25 14. The method of claim 13 wherein the protected peptide or protein further comprises one or more hydroxy amino acid residues wherein the hydroxy group is protected with a t-butyl group.
15. The method of claim 13 wherein the protected peptide or protein further comprises one or 30 more hydroxy amino acid residues wherein the hydroxy group is protected with an optionally substituted benzyl group.

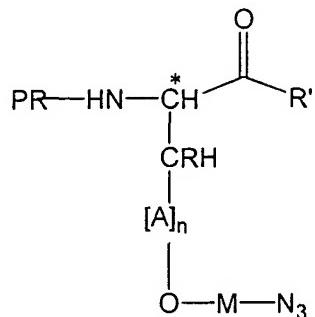
16. The method of claim 1 wherein the peptide or protein is provided by step-wise solid phase peptide synthesis on a resin employing an amino protected tyrosine in which the phenol group is protected with an azidomethylene group to incorporate at least one protected tyrosine residue on the protein or peptide synthesized on the resin.

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17. The method of claim 16 wherein Fmoc-based solid phase peptide synthesis is used to provide the peptide.

18. A protected amino acid useful for synthesis of a selectively derivatized peptide which has
10 the formula:

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and salts thereof wherein:

20 PR is any appropriate amine protecting group wherein the conditions for removal of the protecting group are substantially orthogonal to the conditions for removal of the azide-bearing protecting group;

25 R' is OH, OR, OAr, NH₂, NH(R or Ar), NR₂, N(Ar)₂, a group that generates an activated ester, a halogen, a substituted phenyl group, a halogenated phenyl group, benzotriazol-1-yl, N-hydroxysuccinimido, or 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl ;

30 R is H or alkyl,

A is an optionally substituted phenyl group;

n is 0 or 1; and

M is selected from the group consisting of:

-(CH₂)_m- where m is 1-6;

5 -CH₂-phenyl- wherein the phenyl can be optionally substituted;

-CH₂-phenyl-O- wherein the phenyl can be optionally substituted;

-(CR₂)_m- where m is 1-6, each R selected independently of other R;

-CO-NH-SO₂-CH₂-CH₂-; and

-CO-NH-CH₂-CH₂-.

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19. The protected amino acid of claim 18 wherein PR is acid labile.

20. The protected amino acid of claim 18 wherein PR is base labile.

15 21. The protected amino acid of claim 18 wherein PR is selected from the group consisting of :Boc, Bpoc, Trityl, Fmoc; 2-nitrosulphonyl, dithiasuccinoyl, diphenylphosphinyl, and sulfonyl.

22. The protected amino acid of claim 18 wherein n is 1 and R is H.

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23. The protected amino acid of claim 18 wherein n is 0 and R is CH₃.

24. The protected amino acid of claim 18 wherein n is 0 and R is H.

25 25. The protected amino acid of claim 18 wherein the amino-protecting group is Fmoc.

26. The protected amino acid of claim 25 wherein n is 1 and R is H.

27. The protected amino acid of claim 26 wherein M is -CH₂-.

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28. The protected amino acid of claim 18 wherein M is -CH₂-.

29. The protected amino acid of claim 18 wherein R' is fluoride or chloride.
30. A kit for the synthesis of a derivatized peptide which comprises one or more of the azide-protected amino acids of claim 18.
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31. The kit of claim 30 wherein, in the azide-protected amino acid, PR is acid labile.
32. The kit of claim 30 wherein, in the azide-protected amino acid, PR is base labile.
- 10 33. The kit of claim 30 wherein, in the azide-protected amino acid, PR is selected from the group consisting of :Boc, Bpoc, Trityl, Fmoc, Fmoc; 2-nitrosulphonyl, dithiasuccinoyl, diphenylphosphinyl, and sulfonyl.
34. The kit of claim 30 wherein, in the azide protected amino acid, n is 1 and R is H.
- 15 35. The kit of claim 30 wherein, in the azide protected amino acid, n is 0 and R is CH₃.
36. The kit of claim 30 wherein, in the azide protected amino acid, n is 0 and R is H.
- 20 37. The kit of claim 30 wherein, in the azide protected amino acid, the amino-protecting group is Fmoc.
38. The kit of claim 30 wherein, in the azide protected amino acid, n is 1 and R is H.
- 25 39. The kit of claim 38 wherein, in the azide protected amino acid, M is -CH₂-.
40. The kit of claim 30 wherein, in the azide protected amino acid, M is -CH₂-.
- 30 41. The kit of claim 30 further comprising one or more amino acids for peptide synthesis other than azide-protected hydroxy amino acids wherein said one or more amino acids for peptide synthesis comprise α -amine group protection, optional side-chain protection and

optional carboxy group protection, activation or both as appropriate for use with PR and the azide protecting group of the azide-protected hydroxy amino acids in the kit.

42. The kit of claim 41 wherein, in the azide-protected amino acid, PR is Fmoc.
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43. The kit of claim 41 wherein, in the azide-protected amino acid, PR is Boc.

44. The kit of claim 30 further comprising solid support materials appropriate for conducting peptide synthesis employing the protected amino acid or acids provided in the kit.
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45. The kit of claim 30 further comprising one or more reagents for deprotecting the azide-protected amino acids in the kit.

46. The kit of claim 30 further comprising one or more reagents for sulfation of a deprotected hydroxy amino acid.
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47. The kit of claim 30 further comprising one or more reagents for phosphorylation of a deprotected hydroxy amino acid.

20 48. The kit of claim 30 further comprising one or more reagents for glycosylation of a deprotected hydroxy amino acid.

49. The kit of claim 30 further comprising instructions for conducting peptide synthesis employing the azide-protected amino acids in the kit.
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50. A method for synthesizing a selectively modified peptide or amino acid which comprises the step of synthesizing a selectively-modified peptide employing the kit of claim 30.

51. A method for sulfation of one or more hydroxy amino acid residues in a peptide or protein which comprises the steps of:
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providing a peptide or protein comprising one or more hydroxy amino acid residues wherein at least one of the hydroxy group of at least one of the one or more hydroxy amino acid residues in the polymer is protected with an azide-bearing protecting group; deprotecting the at least one azide-protected hydroxy amino acid residues in the peptide or protein under azide reducing conditions to generate a free hydroxy group; and

sulfating the free hydroxy group generated on deprotection.

52. The method of claim 51 wherein the peptide or protein is a glycopeptide or glycoprotein.

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53. The method of claim 51 wherein the hydroxy amino acid residues are tyrosines.

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54. The method of claim 51 wherein the peptide further comprises one or more hydroxy amino acid residues that are protected with a protecting group that are not cleaved under conditions for azide-bearing protecting group removal.

55. The method of claim 55 wherein the hydroxy amino acid residues are tyrosines.

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56. The method of claim 55 wherein the peptide or protein further comprises one or more tyrosine residues, the phenol group of which is protected with a benzyl group.

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57. The method of claim 50 wherein at least a portion of the peptide or protein is provided by step-wise solid phase peptide synthesis on a resin employing an amine-protected hydroxy amino acid in which the hydroxy group is protected with an azidomethylene group to incorporate at least one azide-protected hydroxy amino acid residue on a peptide synthesized on the resin.

58. The method of claim 57 wherein the amine protection group on the amine-protected hydroxy amino acid is an Fmoc group.

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59. The method of claim 57 wherein the hydroxy amino acid is a tyrosine.

60. The method of claim 59 wherein the amine protection group on the amine-protected tyrosine is an Fmoc group.
- 5 61. The method of claim 57 wherein the azidomethylene protecting group is cleaved prior to cleavage of the peptide from the resin.
- 10 62. The method of claim 61 wherein the resin is a 2-chlorotriyl resin.
63. The method of claim 51 wherein the free hydroxy group is sulfated by DMF.SO₃ in a pyridine/DMF mixture.
- 15 64. The method of claim 63 wherein the free hydroxy group is the free hydroxy group of a tyrosine.
65. The method of claim 51 wherein fewer than all of the hydroxy amino acid residues present in the peptide or protein are sulfated.
66. The method of claim 65 wherein fewer than all of the tyrosine residues present in the peptide or protein are sulfated.